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Medical use

Background of the invention

- 5 The present invention provides compounds which are useful for treating diseases that are related to an abnormal loss of cells.

10 Traumatic, asphyxial, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS) often result in damages to several different cell types. Thus, damages to the brain by trauma, asphyxia, toxins, ischemia or infections frequently cause neurological and cognitive deficits.

15 Perhaps the most severe form of neurodegeneration is that seen after stroke. This form of cerebral ischemia results in the death of neurons, as well as glial cells and vascular elements of the brain. Quite often a stroke results in paralysis, memory loss, and an inability to communicate.

20 Another form of cerebral ischemia that can be quite devastating to important groups of selectively vulnerable neurons, is global ischemia. Global cerebral ischemia is commonly seen in victims of cardiac arrest during the period of time the heart is undergoing fibrillation. Neuronal death from global ischemia is a common occurrence in heart attack victims that undergo cardiac arrest and cardiac arrest is a common occurrence in heart attack patients.

25 Parkinson's disease is a movement disorder in which symptomatology is defined by three cardinal symptoms; tremor at rest, rigidity and akinesia (Fahn, 1989). The disease often causes loss of specific populations of cells and is in particular associated with the specific loss of dopaminergic neurons in the Substantia nigra. The course of the disease is a progressive one. For a long time, anticholinergic drugs were the only effective treatment of parkinsonian symptoms. The beneficial effect of L-3,4-
30 dihydrophenylalanine (L-DOPA) therapy has increased patient's life expectancy to a

significant degree. However, the advanced stage of the disease is dominated by the complications of L-DOPA therapy and lack of L-DOPA responsiveness. A limiting factor in PD therapy is the psychotic potential of many anti-parkinsonian drugs.

- 5 Amyotrophic lateral sclerosis, (ALS), is a chronic progressive degenerative disorder, which, in its classical form, appears sporadically. The most prominent pathological change in ALS patients is a loss of large motoneurons in the motor cortex, brain stem and spinal cord. In motoneuron disease, (e.g. ALS), a degeneration of the central pyramidal, the peripheral motor system or both is the reason for the clinical picture.

10

Another illustration of a degenerative disorder caused by selective loss of a specialized type of neurons is Alzheimer's disease, (AD), which is associated with loss of cholinergic neurons. Cognitive decline is the essential clinical criteria for AD manifested by memory loss, disorientation and the concomitant loss of enjoyment of life associated therewith. Only after death can the diagnosis be confirmed pathologically by the presence of numerous amyloid and neuritic plaques in the brain.

15

Similarly, multiple sclerosis, (MS), is associated with loss of myelin and oligodendrocytes. Additionally, there are many other instances in which CNS injuries or diseases can cause damage to oligodendroglia, astroglia, or neuronal cells.

20

At present, the pharmacological therapy of neurodegenerative disorders is limited to symptomatic treatments that do not alter the course of the underlying disease.

- 25 Meanwhile, because of the current dissatisfaction with the currently marketed treatments for the above-described indications within the affected population, the need continues for safer and better treatments which will either slow the process of neurodegeneration associated with complications or conditions such as focal or global ischemia, ALS, Alzheimer's and Parkinson's disease or even prevent such
- 30 neurodegeneration altogether.

Gastric Inhibitory polypeptide (GIP) is an insulintrophic peptide naturally occurring in human neuroendocrine cells of the small intestine (Buchan A., Polak J., Capella C., Solcia E. and Pearse A., *Histochemistry* 56: 37-44 (1978)). Its primary function is as an incretin, mediating postprandial insulin release from pancreas (Pederson R., Schubert H. and Brown J., *Diabetes* 24: 1050-1056 (1975)); Pederson R. and Brown J., *Endocrinology* 99: 780-785 (1976)).

GIP is a 42 amino acid polypeptide chemically related and showing a structural homology to other members of the secretin-glucagon family of gastrointestinal regulatory polypeptides, including secretin, glucagon, glucagon-like peptide 1 and 2 (GLP 1 and 2), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine (PHI), growth hormone releasing hormone (GHRH) and pituitary adenylyl cyclase-activating polypeptide (PACAP) (Tseng C., Jarboe L., Landau S., Williams E. and Wolfe M., *Proc Natl Acad Sci USA* 90: 1992-1996 (1993)).

Expression of mRNA for the GIP receptor has been reported in the areas of the brain, including hippocampus and olfactory bulb (Usdin T., Mezey E., Button D., Brownstein M. and Bonner T., *Endocrinology* 133: 2861-2870 (1993); Kaplan A. and Vigna S., *Peptides* 15: 297-302 (1994)).

Summary of the invention

The invention relates to a compound, gastric inhibitory peptide (GIP), for the use as a medicament. It further relates to the use of this compound for the preparation of a medicament for the treatment, including veterinary treatment of livestock, of conditions that are characterized by a pathological loss of cells, such as Parkinson's disease, Alzheimer's disease, Stroke, Multiple Sclerosis, stroke, asphyxia or hypoxia, heart failure, heart infarction, diabetes, arthrosis or arthritis, skin disease and burn injuries, liver diseases or failure, muscle diseases or damages, pancreatic dysfunction, inflammatory bowel disease. Also included in the group of diseases are diseases

caused by prions, such as Creutzfeld-Jacob's disease, scrapie and bovine spongiform encefalitis (BSE).

5 It is envisaged that an antagonist against a compound according to the invention may be used as an inhibitor of cell proliferation.

A different condition shown to be susceptible to treatment by compounds according to the invention is obesity.

10 Detailed description

The 42 amino acid gastric inhibitory peptide (GIP) has, apart from its principal insulinotropic effect on pancreas, been reported to have an influence on other systems. It influences, among others, properties of hepatic venous flow have effects on
15 arteries, enhances collagen synthesis in osteoblast-like cells and increases fatty acid synthesis in adipose tissue (Kogire M., Inoue K., Sumi S., Doi R., Yun M., Kaji H. and Tobe T., Dig Dis Sci 37: 1666-1670 (1992); Bollag R., Zhong Q., Philips P., Min L., Zhong L., Cameron R., Mulloy A., Rasmussen H., Qin F., Ding K. and Isales C., Endocrinology 141: 1228-1235 (2000); Hauner H., Glatting G., Kaminska D. and
20 Pfeiffer E., Ann Nutr Metab 32: 282-288 (1988)).

The inventors have shown that GIP is expressed in the mammalian brain. They have further shown that GIP can cause stem cells, progenitor-cells and other cells, especially cells derived from the central nervous system with the potential to generate
25 differentiated cells, such as neurons, astrocytes and/or oligodendrocytes, to proliferate. This suggests that GIP may be of use as a medicament or used in the preparation of a medicament to be used in the treatment of diseases or disorders that are characterized by an abnormal loss of cells. This includes diseases, conditions and/or damages to the central nervous system (CNS) or peripheral nervous system (PNS) that may be caused
30 by trauma, asphyxia, toxins, hypoxia, ischemia, infections or degenerative or metabolic insults resulting in neurological and cognitive deficits. Examples of such

diseases are Parkinson's disease, Alzheimer's disease, stroke, multiple sclerosis, asphyxia or hypoxia, heart failure, heart infarction, diabetes, artrosis or arthritis, skin disease and burn injuries, liver diseases or failure, muscle diseases or damages, cancer, pancreatic dysfunction, inflammatory bowel disease. Also included in the group of

5 diseases are diseases caused by prions, such as Creutzfeld-Jacob's disease, scrapie and bovine spongiform encefalitis (BSE).

In one aspect of the invention, a compound chosen from the group comprising; a polypeptide according to SEQ ID NO 1, fragments, oligomers of fragments, or

10 analogues thereof, such as a compound with at least 80% similarity, preferably at least 90% similarity, more preferably at least 95%, further more preferably at least 96%, even more preferably at least 97%, or most preferably at least 98% similarity to the polypeptide shown in SEQ ID NO 1, more preferably at least 80% identity, preferably at least 90% identity, more preferably at least 95%, further more preferably at least

15 96%, even more preferably at least 97%, or most preferably at least 98%, identity to the polypeptide shown in SEQ ID NO 1, or oligomers of the above mentioned compounds, for medical use.

Alternatively, a compound according to the above is provided for the use in the

20 preparation of a medicament for treating and/or preventing diseases characterized by a pathological loss of cells or loss of control of proliferation of cells. Such diseases may be chosen from the group comprising; Parkinson's disease, Alzheimer's disease, stroke, multiple sclerosis, asphyxia or hypoxia, heart failure, heart infarction, diabetes, arthrosis or arthritis, skin disease and burn injuries, liver diseases or failure, muscle

25 diseases or damages, cancer, pancreatic dysfunction, inflammatory bowel disease. Also included in the group of diseases are diseases caused by prions, such as Creutzfeld-Jacob's disease, scrapie and bovine spongiform encefalitis (BSE). Other diseases or conditions that are susceptible for therapy by these compounds are those that are characterized by an abnormally increased body weight, e.g. obesity.

As is the case with many polypeptides, a specific part of the peptide may be responsible for the activity. Consequently, fragments of the peptide are also believed to be within the scope of the invention. Furthermore, dimers, trimers, tetramers, pentamers or other oligomers of these fragments are within the scope of the invention.

5 Additionally, oligomers of the whole gastric inhibitory peptide are within the scope of the invention. It is well within the capacity of a skilled person to determine, with the help of the description herein, if an analogue, fragment or oligomer thereof has GIP-like activity, or is an antagonist thereof. Fragments of the peptide may be generated in several ways. A convenient way would be to obtain the fragments from a commercial
10 supplier, such as Innovagen, Lund, Sweden.

A preferred compound for medical use is the polypeptide shown in SEQ ID NO 1.

Another aspect of the present invention is the use of a compound according to the
15 above, for the manufacture of a medicament to be used for therapeutic and/or prophylactic treatment of a medical condition in a mammal, wherein said condition is characterized by a pathological loss of cells, preferably where the loss of cells occur in the central or peripheral nervous system. More preferably, such diseases may be chosen from the group comprising; Parkinson's disease, Alzheimer's disease, stroke,
20 multiple sclerosis, asphyxia or hypoxia, heart failure, heart infarction, diabetes, artrosis or arthritis, skin disease and burn injuries, liver diseases or failure, muscle diseases or damages, cancer, pancreatic dysfunction, inflammatory bowel disease. Also included in the group of diseases are diseases caused by prions, such as Creutzfeld-Jacob's disease, scrapie and bovine spongiform encefalitis (BSE).

25 The inventors have shown that GIP has an effect on reducing weight gain. Therefor, in one embodiment of the invention, use of a compound according to the invention is provided for the manufacture of a medicament to be used for the treatment of conditions that are characterized by an abnormally increased body weight, e.g. obesity.
30

In a different aspect, an antagonist against the compounds according to the invention is provided, for medical use. Such an antagonist may be e.g. an antibody against GIP, or analogues thereof.

It is envisaged that an antagonist directed against GIP, such as an antibody, would have the reverse effect, i.e. inhibition of cell proliferation. Consequently, in yet a different aspect, it is provided the use of an antagonist, (e.g. an antibody) against GIP for the preparation of a medicament to be used in the treatment of diseases or disorders characterized by an abnormal proliferation of cells. It is thus envisaged that diseases that are characterized by an abnormal proliferation of cells may be treated by such antagonists. Such antagonists may be e.g. antibodies raised against GIP. Diseases amenable for treatment by the compounds according to the invention can be exemplified by the group comprising the following; melanoma, non-small-cell lung cancer, small-cell lung cancer, lung cancer, hepatocarcinoma, retinoblastoma, astrocytoma, glioblastoma, leukemia, neuroblastoma, cancer in the gum, tongue, head, neck, breast, pancreas, prostate, kidney, liver, bone, thyroid, testicle, ovary, mesothelia, cervix, gastrointestinal tract, lymphoma, brain, colon, sarcoma or bladder. The cancer may include a tumor comprised of tumor cells. In other embodiments, the hyperproliferative disease is rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, leiomyomas, adenomas, lipomas. Hemangiomas, fibromas, vascular occlusion, retinosis, atherosclerosis, pre-neoplastic lesions, (such as adenomatous hyperplasia and prostatic intraepithelial neoplasia), carcinoma in situ, oral hairy leukoplakia, benign prostatic hyperplasia, or psoriasis. Consequently, the invention provides the use of a compound having an antagonistic effect on GIP-activity for the manufacture of a medicament to be used in the treatment of conditions characterized by an abnormal proliferation of cells.

In a further aspect, it is provided the use of a compound having an antagonistic effect on GIP-activity for the manufacture of a medicament to be used in the treatment of conditions characterized by abnormally low body weight in a mammal.

Even though the compounds according to the invention are suitable for treating any mammal, a preferred subject is a human.

5 In a different aspect, it is provided the use of a compound according to the invention, (i.e. a compound having GIP-activity) for lowering the body weight of a human for cosmetic purposes.

10 In one aspect, the present invention provides the use of a compound having GIP activity for the manufacture of a medicament to be used for therapeutic and/or prophylactic treatment of a medical condition in a mammal, wherein said condition is characterized by a pathological loss of cells, comprising pathological degeneration, loss of ability and/or loss of control of regeneration of; a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

15

One example of a degenerative insult to the CNS is Parkinson's disease. This disease often causes loss of specific populations of cells and is in particular associated with the specific loss of dopaminergic neurons in the Substantia nigra. Parkinson's disease is characterized by reduced range and velocity of movements. In motoneuron disease, 20 (for example, amyotrophic lateral sclerosis, ALS), a degeneration of the central pyramidal, the peripheral motor system or both is the reason for the clinical picture.

Similarly, multiple sclerosis is associated with loss of myelin and oligodendrocytes.

25 Another illustration of a degenerative disorder caused by selective loss of a specialized type of neurons is Alzheimer's disease, which is associated with loss of cholinergic neurons. In Alzheimer's disease, cognitive impairment is the cardinal clinical symptom. Additionally, there are many other instances in which CNS injuries or diseases can cause damage to, or loss of, oligodendroglia, astroglia, or neuronal cells.

30 The inventors show that the compounds according to the present invention are effective for treatment of any pathological condition affecting abnormal gain and/or

loss of differentiated cells or tissues i.e. chondrocytes, cardiomyocytes, oligodendroglia, astroglia, neuronal cells, different types of epithelium, endothelium, skin, blood, liver, kidney, bone, connective tissue, lung tissue, exocrine gland tissue and/or endocrine gland tissue.

The invention relates to a compound, gastric inhibitory peptide (GIP), for the use as a medicament. It further relates to the use of this compound for the preparation of a medicament for the treatment, including veterinary treatment of livestock, of conditions that are characterized by a pathological loss of cells, such as Parkinson's

Administration

weight. It is understood that the ordinarily skilled physician or veterinarian will readily be able to determine and prescribe the amount of the compound effective for the desired prophylactic or therapeutic treatment.

5 In so proceeding, the physician or veterinarian may employ an intravenous bolus followed by an intravenous infusion and repeated administrations, as considered appropriate. In the methods of the present invention, the compounds may be administered, for example, orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, sublingually, vaginally, intraventricularly, or via an implanted
10 reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Parenteral includes, but is not limited to, the following examples of administration: intravenous, subcutaneous, intramuscular, intraspinal, intraosseous, intraperitoneal,
15 intrathecal, intraventricular, intrasternal or intracranial injection and infusion techniques, such as by subdural pump. Invasive techniques are preferred, particularly direct administration to damaged neuronal tissue. While it is possible for the compound of formula I to be administered alone, it is preferable to provide it as a part of a pharmaceutical formulation.

20 To be effective therapeutically as central nervous system targets, the compounds used in the methods of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot penetrate the blood-brain barrier, however, can still be effectively administered by an intraventricular route.

25 The compounds used in the methods of the present invention may be administered by a single dose or by multiple discrete doses.

For the methods of the present invention, any effective administration regimen
30 regulating the timing and sequence of doses may be used. Doses of the compounds preferably include pharmaceutical dosage units comprising an efficacious quantity of

active compound. By an efficacious quantity is meant a quantity sufficient to inhibit induce proliferation of cells and/or derive the desired beneficial effects therefrom through administration of one or more of the pharmaceutical dosage units. In a particularly preferred embodiment, the dose is sufficient to prevent or reduce the effects of neurodegenerative diseases.

An exemplary daily dosage unit for a vertebrate host comprises an amount of from about 0.001 mg/kg to about 50 mg/kg. Typically, dosage levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex, and diet of the patient; the time of administration; the rate of excretion; any combination of the compound with other drugs; the severity of the particular disease being treated; and the form and route of administration.

Typically, in vitro dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models can also be helpful. The considerations for determining the proper dose levels are well-known in the art.

In methods of treating nervous insult (particularly acute ischemic stroke and global ischemia caused by drowning or head trauma), the compounds of the invention can be co-administered with one or more other therapeutic agents, preferably agents which can reduce the risk of stroke (such as aspirin) and, more preferably, agents which can reduce the risk of a second ischemic event (such as ticlopidine).

To kill cells, inhibit cell growth, inhibit metastasis, decrease tumor or tissue size and otherwise reverse or reduce the malignant phenotype of tumor cells, using the methods and compositions of the present invention, one would generally contact a hyperproliferative cell with the therapeutic expression construct. The routes of administration will vary, naturally, with the location and nature of the lesion, and include, e.g. intradermal, transdermal, parenteral, intravenous, intramuscular,

intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intratumoral, perfusion, lavage, direct injection, and oral administration and formulation.

Intratumoral injection, or injection into the tumor vasculature is specifically contemplated for discrete, solid, accessible tumors. Local, regional or systemic administration also may be appropriate. For tumors of >4 cm, the volume to be administered will be about 4-10 ml (preferably 10 ml), while for tumors of <4 cm, a volume of about 1-3 ml will be used (preferably 3 ml). Multiple injections delivered as a single dose comprise about 0.1 to about 0.5 ml volumes. The compound according to the invention may advantageously be contacted by administering multiple injections to the tumor, spaced at approximately 1-cm intervals.

In the case of surgical intervention, the present invention may be used preoperatively, to render an inoperable tumor subject to resection. Alternatively, the present invention may be used at the time of surgery, and/or thereafter, to treat residual or metastatic disease. The perfusion may be continued post-resection, for example, by leaving a catheter implanted at the site of the surgery. Periodic post-surgical treatment is also envisioned.

Continuous administration also may be applied where appropriate, for example, where a tumor is excised and the tumor bed is treated to eliminate residual, microscopic disease. Delivery *via* syringe or catheterization is preferred. Such continuous perfusion may take place for a period from about 1-2 hours, to about 2-6 hours, to about 6-12 hours, to about 12-24 hours, to about 1-2 days, to about 1-2 wk or longer following the initiation of treatment. Generally, the dose of the therapeutic composition *via* continuous perfusion will be equivalent to that given by a single or multiple injections, adjusted over a period of time during which the perfusion occurs. It is further contemplated that limb perfusion may be used to administer therapeutic compositions of the present invention, particularly in the treatment of melanomas and sarcomas.

Treatment regimens may vary as well, and often depend on tumor type, tumor location, disease progression, and health and age of the patient. Obviously, certain types of tumor will require more aggressive treatment, while at the same time, certain patients cannot tolerate more taxing protocols. The clinician will be best suited to make such decisions based on the known efficacy and toxicity (if any) of the therapeutic formulations.

In certain embodiments, the tumor being treated may not, at least initially, be resectable. Treatments with therapeutic viral constructs may increase the resectability of the tumor due to shrinkage at the margins or by elimination of certain particularly invasive portions. Following treatments, resection may be possible. Additional treatments subsequent to resection will serve to eliminate microscopic residual disease at the tumor site.

A typical course of treatment, for a primary tumor or a post-excision tumor bed, will involve multiple doses. Typical primary tumor treatment involves a 6-dose application over a two-week period. The two-week regimen may be repeated one, two, three, four, five, six or more times. During a course of treatment, the need to complete the planned dosings may be re-evaluated.

The treatments may include various "unit doses." Unit dose is defined as containing a predetermined-quantity of the therapeutic composition. The quantity to be administered, and the particular route and formulation, are within the skill of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time. Unit dose of the present invention may conveniently be described in terms of mg/kg body weight.

For treating diseases such as psoriasis, the compound according to the invention is preferably administered as a lotion, cream, or any other composition suitable for administering a medicament on skin.

The compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3 and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988 ISBN 0-8247-2800-9.

The choice of pharmaceutically acceptable excipients in a composition for use according to the invention and the optimum concentration thereof cannot generally be predicted and must be determined on the basis of an experimental determination thereof. Also whether a pharmaceutically acceptable excipient is suitable for use in a pharmaceutical composition is generally dependent on which kind of dosage form is chosen. However, a person skilled in the art of pharmaceutical formulation can find guidance in e.g., "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3.

Definitions

It is to be understood that the terminology used herein is for the purpose of describing particular embodiments and aspects of the invention only, and is not intended to limit the scope of the invention.

Throughout this specification and the claims, the words "comprises" and "comprising" are used in a non-exclusive sense.

It should be noted that, as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

- 5 As defined herein, the terms "GIP" or "gastric inhibitory peptide" are meant to refer to the polypeptide shown in SEQ ID NO 1.

As defined herein, the terms "GIP-activity" or "GIP-like activity" relate to the activity of GIP which induces cell-proliferation, and/or the activity which reduces weight gain.

- 10 An antagonist against the GIP-activity would consequently reduce the cell-proliferating activity and/or increase weight gain.

The term "antagonistic" effect as defined herein, is meant that the effect is to counter the proliferative effect of GIP on cells, or alternatively, to counter the weight reducing effect of GIP, (i.e. inducing weight gain).

- 15

As defined herein, the terms "similarity" or "similar substitutions" mean that chemically similar amino acids replace each other. For example, the basic residues Lys and Arg are considered chemically similar and often replace each other, as do the acidic residues Asp and Glu, the hydroxyl residues Ser and Thr, the aromatic residues Tyr, Phe and Trp, and the non-polar residues Ala, Val, Ile, Leu and Met. Similarity is measured by dividing the number of similar residues by the total number of residues and multiplying the product by 100 to achieve a percentage.

25

By "identity" is meant a property of sequences that measures their similarity or relationship. Identity is measured by dividing the number of identical residues by the total number of residues and multiplying the product by 100 to achieve a percentage. Thus, two copies of exactly the same sequence have 100% identity, but sequences that are less highly conserved and have deletions, additions, or replacements may have a lower degree of identity. Those skilled in the art will recognize that several computer

30

programs, such as those that employ algorithms such as BLAST (Basic Local Alignment Search Tool, Altschul et al. (1993) J. Mol. Biol. 215:403-410) are available for determining sequence identity.

- 5 As defined herein, the term "analogue", in the context of the GIP polypeptide, is meant a polypeptide in which one or more amino acids are replaced by a different, natural or artificial, amino acid. Also included are variants of GIP in which deletions, substitutions, additions or repeats of one or more amino acids have been introduced. Furthermore, fragments of the peptide, or oligomers of these fragments are included.

10

The term "neuroprotective" refers to the effect of reducing, arresting or ameliorating nervous insult, and protecting, resuscitating, or reviving nervous tissue that has suffered nervous insult.

- 15 As defined herein, the term "pathological degeneration" refers to a loss of ability and/or loss of control of regeneration of; a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

- 20 The term "ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Global ischemia occurs when blood flow to the entire brain ceases for a period of time. Global ischemia may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply. Focal ischemia may result from thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumor. Even if transient, both global and focal ischemia can cause widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following the cessation of blood flow to the brain.

30

the term "neurodegenerative diseases" includes Alzheimer's disease, Parkinson's disease and diseases that result from ischemia and reperfusion injury and includes toxicity, such as seen in vascular stroke and global and focal ischemia, as well as ischemia.

The term "nervous insult" refers to any damage to nervous tissue and any disability or resulting therefrom. The cause of nervous insult may be metabolic, toxic, hypoxic, iatrogenic, thermal or chemical, and includes without limitation, ischemia, hypoxia, cerebrovascular accident, trauma, surgery, pressure, mass effect, hemorrhage, radiation, vasospasm, neurodegenerative disease, infection, Parkinson's disease, amyotrophic lateral sclerosis (ALS), myelination/demyelination process, dementia, cognitive disorder, glutamate abnormality and secondary effects thereof.

The term "preventing neurodegeneration" includes the ability to prevent neurodegeneration in patients diagnosed with a neurodegenerative disease or who are at risk of developing a neurodegenerative disease. The term also encompasses preventing further neurodegeneration in patients who are already suffering from or exhibiting symptoms of a neurodegenerative disease.

As defined herein, the terms "treating", "treat" or "treatment" include preventative (prophylactic) and palliative treatment.

The terms "treating", "treat", or "treatment" refer to:

- preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition, but has not yet been diagnosed as having it;
- inhibiting the disease, disorder or condition, i.e., arresting its development; and
- relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

defined herein, the term "abnormal proliferation" is meant that cells are
 erating faster than usual. Abnormal proliferation may result in cancer or tumors,
 er diseases such as psoriasis or acne. These diseases are well known and a
 it suffering from any of these diseases can be properly diagnosed by a person
 d in the art.

defined herein, the term "therapeutic treatment" in the context of conditions such as
 ty or feeding disorders, is used where the patient is diagnosed with obesity or
 xia.

defined herein, the term "obesity" is defined as the condition in which a patient has
 ly mass index (BMI, calculated as $\text{weight (in kg)} / (\text{length (in m)})^2 \text{ (kg/m}^2\text{))}$ above
 normally diagnosed as being obese.

defined herein, the term "cosmetic purpose" is used when GIP or an analogue
 of is used for treating a patient with a BMI below 30.

term "pathological loss and/or gain of cells" is in the present context used to
 ribe the common technical feature of a wide variety of medical conditions and
 rders. The described conditions and disorders are hereby characterized by
 laying pathological degeneration of, loss of ability of regeneration of and/or loss
 ontrol of regeneration of a differentiated cell and/or tissue, an embryonic stem cell,
 dult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor

example, a patient with a BMI below 16 is considered to be anorexic or grossly
 erweight and may be treated with an antagonist of GIP with the purpose of
 ucing weight gain.

used herein the term "mammal" is meant to refer to any mammal, including, for
 mple, primates such as humans and monkeys. Examples of other mammals

comprised herein are rabbits, dogs, cats, and livestock such as cattle, goats, sheep and horses.

Figure legends

In the examples below, reference is made to the appended drawings on which:

Figure 1

This figure illustrates the effect of different doses of GIP on cells, measured as DNA content in adult derived hippocampal stem cell cultures after incubation with different concentrations of GIP compared with untreated control cultures and cultures treated with 20ng/ml of bFGF. (* $P < 0.05$).

Figure 2

The proliferative effects of GIP on progenitor cells in the adult dentate gyrus is illustrated. The number of BrdU immunoreactive cells were counted after intracerebroventricular infusion of GIP compared with vehicle treated controls. (* $P < 0.05$). The density of BrdU-positive cells (cells per cubic millimeter of sample volume) in the granule cell layer was determined stereologically. GIP-treated animals ($n = 5$) exhibited 86% more BrdU-immunoreactive cells than animals treated with PBS (0.1 M; $n = 6$). Values are means \pm SEM (each experiment in the cell cultures represents the mean of four different culture wells). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (one-way ANOVA followed by Fisher's *post hoc*).

Figure 3

The effect of GIP on weight gain in rats.

Examples

The techniques and reagents used in the examples described below are well known to a person skilled in the art. Polynucleotides are written in 5' to 3' direction. Reagents are commercially available. The animal experiments can be justified on the basis that potential suffering of the animals is outweighed by the potential benefit achieved in treating the above mentioned disabling diseases. Experimental protocols were approved by the Animal Ethics Committee of Göteborg University

Animals. All animals were supplied by Møllegaard Breeding Center, Ejby, Denmark. Animals were maintained under standard conditions of temperature (24-26 °C) and humidity (50-60%) and had access to water and standard chow *ad libitum*.

Examples

Example 1

Proliferation measured as DNA content in adult derived hippocampal stem cell cultures.

Quantification of DNA content in adult derived stem cells. The quantification of DNA content was performed according to manufacturer's instruction (Molecular Probes, OR) using the Cyquant DNA assay. Adult derived hippocampal stem cells were cultured at 0.2×10^4 cells/cm² in 24-well plates coated with polyornithine and thereafter laminin. The cells were cultured in N2 medium plus bFGF (basic fibroblast growth factor which is a well known inducer of stem cell proliferation) for two days, followed by N2 without bFGF for two days. Cells were cultured without FGF-2 as a negative control and given a basal level of 100 %. Thereafter the cells were incubated in N2 medium without bFGF with GIP-peptide for 48 h, washed once in PBS and stored in -80 C freezer. Cells were thawed and resuspended in lysis buffer containing EDTA (1 mM), and DNase-free RNase (Sigma) was then added to a final concentration of 1 µg/ml for 1 h at room temperature. Cyquant dye was added for 5 min at room temperature and DNA concentration measured by fluorescence spectroscopy at 480 nm excitation and 520 nm emission wavelengths using a Tecan

Genobios microplate reader. DNA content is calculated as a percentage of content obtained from cells grown without FGF-2. DNA content in cultured adult hippocampal progenitor cells was measured following incubation with different concentrations of GIP ($n = 8$). GIP increases proliferation in a dose-dependent manner and has the greatest effect at a concentration of 1 nM. Results are shown in fig. 1. Statistical analysis between control and each group were using Student's t-test. The results are shown in figure 1. It is evident that cells treated with GIP show an increase in proliferation. DNA content was measured in cultured adult hippocampal progenitor cells following incubation with different concentrations of GIP for 48 h. The diagram shows that GIP increased proliferation in a dose-dependent manner with the greatest effect at a concentration of 1 nM GIP.

Example 2

15 Proliferative effects of GIP on progenitor cells in the adult dentate gyrus.

Adult male Sprague-Dawley rats (weighing 260-280 g) were anaesthetized with isoflurane and the animals were intubated and mechanically ventilated with 3 % isoflurane in an O_2/N_2O mix. Rats were placed in a stereotaxic frame and the skull exposed. An infusion cannula connected to an osmotic pump (Alzet brain infusion kit II and Alzet 2001 osmotic pump, Alza Scientific Products, Palo Alto, CA) was placed in the ventricle (0.3 mm posterior from Bregma along the midline, 5 mm below skull surface) and secured with dental cement. The pump was placed in a subcutaneous pocket in the mid-scapular region, the wound closed and the rat allowed to recover. Each rat was infused (1 μ l/hr) for 5 days with either GIP (1.92 nmol/day) dissolved in 0.1 M PBS ($n = 5$) or 0.1 M PBS only ($n = 6$). All animals received a single daily intraperitoneal injection of Bromodeoxyuridine (BrdU; 50 mg/kg of body weight; Boehringer Mannheim; Scandinavia AB, Bromma, Sweden). On the sixth day the animals were anaesthetised briefly with isoflurane and decapitated. The brain was removed and postfixed. All brains throughout the study were sectioned coronary (40 μ m) through the entire hippocampus on a freezing microtome. Staining was performed

on free-floating 40 μm sections pretreated with 0.6% H_2O_2 in tris-buffered saline for 30 min to block endogenous peroxidase activity. To ensure detection of BrdU-labeled nuclei, we denatured the DNA before incubation with mouse anti-BrdU antibody (1:400, Boeringer Mannheim). DNA denaturation was performed in the following manner: Tissue was incubated in 50% formamide and 2 x SSC (1 x SSC, 0.3 M NaCl and 0.03 M sodium citrate) for 2 hr at 65 $^\circ\text{C}$, rinsed for 15 min in 2 x SSC, incubated again for 30 min in 2 M HCl at 37 $^\circ\text{C}$ followed by an additional 10 min rinse in 0.1 M boric acid at pH 8.5. The tissue was then rinsed several times in TBS, followed by incubation in TBS-TS (0.25% Triton-X and 3% normal donkey serum in TBS) for 30 min and then with primary antibody in TBS-TS overnight at 4 $^\circ\text{C}$. The next day, tissue sections were incubated for 2 hr with biotinylated horse anti-mouse IgG (1:169) secondary antibodies (Vector Laboratories, Burlingame, CA) and rinsed in TBS. Avidin-biotin-peroxidase complex was applied for 1 hr before 8 min peroxidase detection (using 0.25 mg/ml diaminobenzidine, 0.01% H_2O_2 and 0.04% NiCl). For each animal, the total number of BrdU-positive cells in the granule cell layer, including the subgranular layer, and their corresponding sample volume were determined in 12 immunoperoxidase-stained, 40- μm -thick coronal sections taken 240 μm apart. Cells were disregarded that were sharp in focus in the uppermost focal plane (optical dissector principle). All slides were coded before analysis. The code was not broken until the analysis was completed. The cross sectional areas were obtained using a CCD camera linked to a digital imaging system (Nikon, Sweden). Results are expressed as BrdU-positive cells per sample volume. All values are expressed as the means \pm SEM. Comparisons between groups were made using one-way ANOVA followed by a Fisher's *post hoc*, when appropriate throughout the study. A *p*-value < 0.05 was considered statistically significant. As can be seen in figure 2, animals treated with GIP show an increase in cell proliferation.

Example 3

Demonstration of GIP:s effect on weight gain.

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- The diagrams illustrate the steps of a bubble sort algorithm on the array [1, 2, 3, 4, 5, 6, 7, 8, 9, 10].
- Diagram 1: Initial array: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.
 - Diagram 2: Comparison between 1 and 2. Since 1 < 2, no swap occurs.
 - Diagram 3: Comparison between 2 and 3. Since 2 < 3, no swap occurs.
 - Diagram 4: Comparison between 3 and 4. Since 3 < 4, no swap occurs.
 - Diagram 5: Comparison between 4 and 5. Since 4 < 5, no swap occurs.
 - Diagram 6: Comparison between 5 and 6. Since 5 < 6, no swap occurs.
 - Diagram 7: Comparison between 6 and 7. Since 6 < 7, no swap occurs.
 - Diagram 8: Comparison between 7 and 8. Since 7 < 8, no swap occurs.
 - Diagram 9: Comparison between 8 and 9. Since 8 < 9, no swap occurs.
 - Diagram 10: Comparison between 9 and 10. Since 9 < 10, no swap occurs.
- The final array is [1, 2, 3, 4, 5, 6, 7, 8, 9, 10], which is already sorted.

Claims

1. A compound having GIP-activity, chosen from the group comprising; analogues of
5 the polypeptide according to SEQ ID NO 1, oligomers thereof, fragments,
oligomers of fragments, or analogues thereof, for medical use.
2. A compound according to claim 1 in which the compound has 80% similarity,
preferably 90% similarity, more preferably 95%, further more preferably 96%,
10 even more preferably 97%, or most preferably 98% similarity to the polypeptide
shown in SEQ ID NO 1, for medical use.
3. A compound according to claim 1 in which the compound has 80% identity,
preferably 90% identity, more preferably 95%, further more preferably 96%, even
15 more preferably 97%, or most preferably 98%, identity to the polypeptide shown in
SEQ ID NO 1, for medical use.
4. A compound according to claim 1 in which the compound is the polypeptide
shown in SEQ ID NO 1, for medical use.
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5. Use of a compound according to claim 1-4 for the manufacture of a medicament to
be used for therapeutic and/or prophylactic treatment of a medical condition in a
mammal, wherein said condition is characterized by a pathological loss of cells,
- 25 6. Use according to claim 5, wherein the condition to be treated is located to the
central or peripheral nervous system.
7. Use according to claim 5, wherein the condition to be treated is caused by loss of
cells.
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8. Use according to claim 5, wherein the condition to be treated is obesity.

9. An antagonist against GIP for medical use.

5 10. An antagonist according to claim 9, in which the antagonist is an antibody raised against GIP, for medical use.

10 11. Use of an antagonist according to claim 9 or 10, for the preparation of a medicament to be used in the treatment of diseases or disorders characterized by an abnormal proliferation of cells.

12. Use of an antagonist against GIP for the manufacture of a medicament to be used in the treatment of conditions characterized by abnormally low body weight in a mammal.

15 13. Use according to any of the above claims in which the patient is a human.

14. Use of a compound according to claims 1-4, for lowering the body weight of a human for cosmetic purposes.

20 15. A pharmaceutical composition comprising a compound according any of claims 1-14, optionally together with pharmaceutically acceptable excipients and additives.

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Abstract

The invention relates to a compound, gastric inhibitory peptide (GIP), for the use as a medicament. It further relates to the use of this compound for the preparation of a medicament for the treatment, including veterinary treatment of livestock, of conditions that are characterized by a pathological loss of cells, such as Parkinson's disease, Alzheimer's disease, Stroke, Multiple Sclerosis, stroke, asphyxia or hypoxia, heart failure, heart infarction, diabetes, arthrosis or arthritis, skin disease and burn injuries, liver diseases or failure, muscle diseases or damages, pancreatic dysfunction, inflammatory bowel disease. Also included in the group of diseases are diseases caused by prions, such as Creutzfeld-Jacob's disease, scrapie and bovine spongiform encephalitis (BSE).

FIG 1

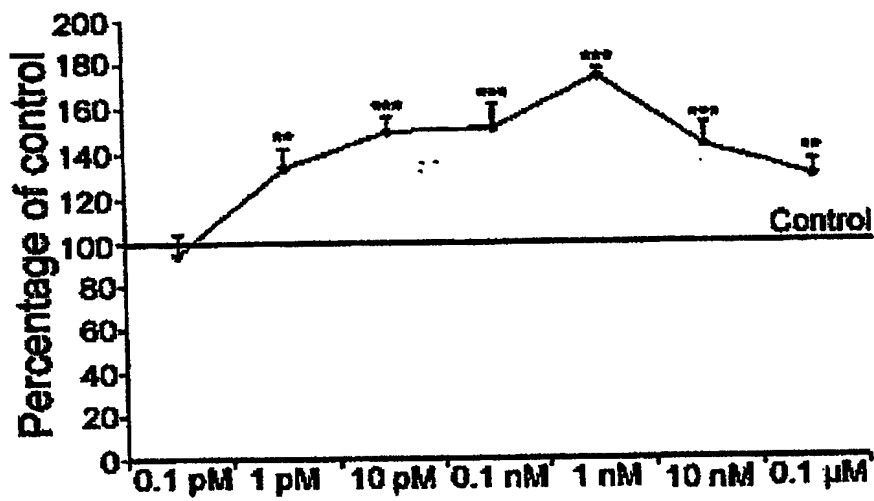
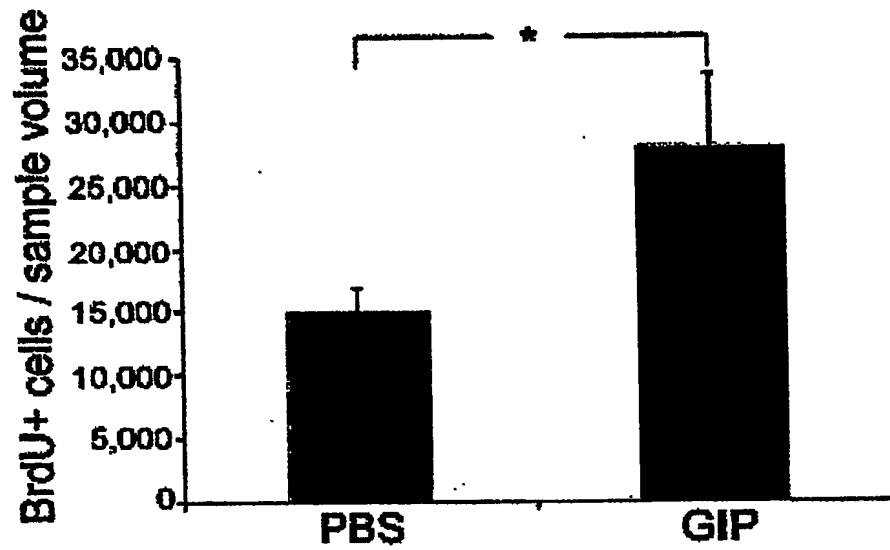


FIG 2



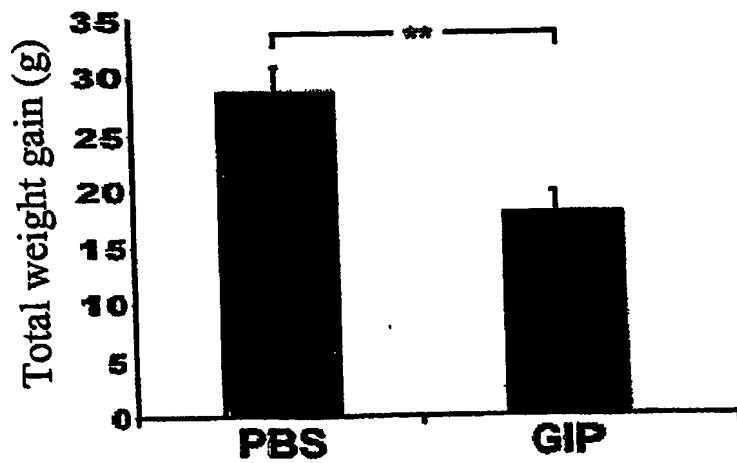


Figure 3

SEQUENCE LISTING

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20

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PRV 020811

Lys Asn Asp Trp Lys His Asn Ile Thr Gln

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